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Review on the start-up experiences of continuous fermentative hydrogen producing bioreactors

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The start-up of continuous biohydrogen fermentations is a complex procedure and a key to acceptable hydrogen production performance and successful long-term operation. In this review article, the experiences gained and lessons learned from relevant literature studies dealing with various aspects of H_2 producing bioreactor start-up are comprehensively surveyed. Firstly, the importance of H_2-forming biosystem start-up including its main steps is outlined. Afterwards, the role of main influencing factors and methods (e.g. strain selection, seed pretreatment and inocula stimulation, switch-over time, bioreactor design, operating conditions) in avoiding the deterioration of starting a reactor is analyzed and presented in detail. Finally, the so far suggested applicable start-up strategies and the corresponding findings are critically discussed pointing out the advantages and disadvantages of each strategy.

Contents

1. Introduction

Hydrogen is an emerging candidate among the various alternative energy carriers. H_2 is believed to help the transition of current fossil-based economy to a renewable-based one [1], however, only if it is derived by sustainable processes. Though H_2 can be prepared by many conventional and mature methods (e.g. steam reformation of hydrocarbons), environmental-friendly methods such as with biological routes are required and still subjects to extensive research [2].

Nowadays, microbiologically produced hydrogen is recognized as an emerging way ahead, especially when formed via dark fermentation because of its inherent advantages such as relatively low energy demand (attributed to the gentle reaction conditions), the usability of wide range of feedstocks e.g. derivates of biomass, waste streams and agricultural residues [3–5], and the possibility to integrate with other e.g. membrane-based processes in order to accomplish the sufficient reuse of hydrogen producing cells [6] or to upgrade bioH_2 [7–9] so that it could be a viable feedstock in energy efficient fuel cells.

Nevertheless, additional efforts are still essential to make biohydrogen generation more attractive. From practical aspect, the two major criteria to be considered are H_2 production yields and rates. As a result of the investigations in the past decades, several factors were identified that significantly affect the main

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technological indicators mentioned above. Among them, bioreactor configuration and operation are apparent ones [10]. Regardless the type of the fermenter, it can be concluded that feasible biohydrogen fermentation should be conducted in continuous rather than batch systems [11], e.g. due to higher expectable process efficiencies.

The establishment of continuous flow bioreactors usually starts in batch mode, and it is to note that successful transition and reliable, long-term operation is highly influenced by the start-up strategy applied [12–14]. However, up to the authors' best knowledge, there is no recent review paper comprehensively surveying start-up experiences in continuous hydrogen producing bioreactors. Hence, in this work, the experiences gained and lessons learned from batch to continuous shifts are reviewed and suggestions are given to achieve proper continuous operation.

2. The role of start-up in the efficacy of continuous hydrogen production

Process instability is a frequently observed drawback in fermentative H_2 production [15] that could be attributed to multiple reasons as specified later in this paper. In fact, beyond steady-state operational parameters and medium composition, stable and continuous bioreactor operation to obtain acceptable hydrogen production performance is strongly dependent on the start-up phase [16]. It could involve the following steps:

– Selection of the hydrogen producing biocatalysts.
– Enhancement and acclimatization of H_2-forming strains to fermentation circumstances.
– System transition until steady-state is reached.

These steps in a line require great attention and comprehensive control in terms of environmental and operational circumstances to develop robustful H_2 fermenting culture [17]. Otherwise, starting a reactor may easily be deteriorated e.g. due to the insufficient growth and H_2 production capacity of microorganisms. Such bottlenecks can be avoided or at least mitigated by properly designed start-up strategy.

In the next sections of the paper, the aforementioned parts of the continuous dark fermentative bioreactor establishment are outlined and discussed in details.

3. Factors affecting the initiation of continuous H_2 fermenters

3.1. H_2 producing strains

Fermentative biohydrogen generation can be realized either by pure cultures [18] such as *Escherichia coli* [19,20] or mixed bacterial consortia [21,22] and both have their own benefits. For example, cultures of pure isolates may be easier to control but need constantly sterile environment to prevent contamination that is difficult and costly to maintain out of laboratories. Considering their application in a non-sterile environment, pure cultures may be used in the bioaugmentation of diverse H_2 producing population to attain better gas turnouts [23]. The restrictions of sterility criteria are the main reasons why mixed bacterial communities are preferred to their pure counterparts in real-case, scaled-up applications.

3.2. Pretreatment and stimulation

Conceptually, anaerobic, mixed H_2-producing consortia (e.g. in sewage sludge, biogas plants, etc.) are built up by co-existing and synergic species [24]. However, in most cases, they naturally occur together with H_2-consumer microorganism such as methanogenic archaea, homoacetogenic (producing acetate from CO_2 and H_2)-, lactic- and propionic acid bacteria which must certainly be suppressed or more preferentially totally eliminated [25]. As a consequence, regardless the source of mixed inocula, it should undergo initial pretreatment in order to select the desired whole cell biocatalysts. For such purposes, a lot of tools have been developed based on heat shock, addition of chemicals, swinging the oxidation–reduction potential (ORP) e.g. by aeration, high energy irradiation, alteration of pH, freezing and thawing [26–28]. These pretreatment techniques exploit the distinct sensitivity of strains present in the mixture and in general could provide a satisfactory starter culture to be used as seed inocula for subsequent biohydrogen fermentation. In other words, these procedures aim to eliminate hydrogen-consuming vegetative cells and on the other hand, are devoted to enhance acidogenic- and often sporulative H_2-forming cells [29].

Although culture pretreatments can effectively suppress undesired microbiological activity, they may also reduce the number of indigenous H_2-former bacteria, especially the ones with low stress tolerance. For these reasons, as a next step after culture pretreatment, treated inocula should be submitted to stimulative environment (e.g. to a batch reactor) to let the microbes proliferate so that a reasonable amount of active cellmass can be accumulated, harvested and further applied. Also, batch cultivation can play a role to help biofilm development on carrier materials (e.g. powdered- and granulated activated carbon) if an immobilized, continuous H_2 production system is to be implemented [30,31].

According to literature reports, pretreated inocula are more often than not dominated by spore-forming and robust H_2-producer species such as *Clostridium* sp. [13]; however some organisms of no utility (e.g. propionic acid and homoacetogenic bacteria) may also survive and reclaim their niche over time [21,25]. Changes in the microbial background can be revealed by the modern technical apparatus of molecular biology [32,33].

Furthermore, it is presumable that the age of the seed source – most commonly sewage or biogas (anerobic fermenter) sludge as suggested by Tables 1 and 2 – may also be a factor to take into account. It is assumable that the microbial community structure of anaerobic mixed cultures varies constantly during storage due to changes (e.g. concentration differences) within micro-environments. Consequently, aging of an anaerobic seed culture over time can result in the variation of the obtainable bacterial populations and their activity. Thus, it might lead to alterations in the attainable biohydrogen performances even though standardized, identical pretreatment conditions are ensured time after time to prepare H_2 producing inocula.

Moreover, beyond the goal of activating H_2-producer organisms [16,34,35], preliminary cultivation – mostly in batch – may also serve as a tool to acclimatize the microflora to certain substrates and their loadings e.g. to overcome inhibitory effect [36], which will induce a dynamic competition between the various groups of bacteria. Although batch-continuous start-up strategy was proposed by various authors to follow (for examples, please refer to Tables 1 and 2), some researchers reported adequate start-up directly in continuous operation [37–41].

The advantage of this strategy lacking initial discontinuous cell growth might be that in batch operation the nutrient concentrations as the time passes, especially at the end hours of the cycle, are insufficiently low and consequently a shift in the dominant strains could occur, depressing H_2 production [12]. During careful continuous adaptation, broth is constantly supplemented and such disadvantageous phenomenon may be avoided. Moreover, continuous (hydraulic detention time influenced) acclimatization strategy encompasses the so-called biokinetic control which causes the

Table 1
Start-up experiences during H_2 fermentation in CSTR.

Reactor type	Inoculum	Inoculum Pretreatment	Substrate	pH	*T* (°C)	Start-up experiences	Reference
CSTR	5 Different thermophilic sludge	–	Starch	N.C.	55	Continuous feeding was started after obtaining exponential growth phase in batch operation. Stable hydrogen production was attained in less than 30 days of start-up	[70]
CSTR	Indigenous microflora of substrate	–	Sweet sorghum extract	3.5–6.5	35C	24 h in batch mode to activate the indigenous microflora contained in substrate	[74]
CSTR	Anaerobic digester sludge	Heat shock (85 °C, 45 min)	Cheese whey wastewater	5.5	55	2 days in batch mode, conversion to continuous operation when hydrogen production reached its peak value	[75]
CSTR	*E. coli*	–	Na-formate	6.5	37	Batch operation until exponential growth phase took place	[20]
CSTR	Anaerobic granular sludge	Heat shock (boiling for 40 min)	Cheese whey	7.5	37	Batch operation for 12 h	[49]
CSTR	Digester sludge	N.M.	Cellulose	N.C.	70	90 days until steady-state	[72]
CSTR	Waste activated sludge	Heat shock (70 °C, 30 min)	Glucose	5.5–6.5	37	15 h in batch mode, 10 days to reach steady-state	[39]
CSTR	Anaerobic digester sludge	N.M.	Sugarbeet water extract	5.2	32	Continuous operation was commenced once significant hydrogen production occurred	[76]
CSTR	Anaerobic digester sludge	Heat shock (90 °C, 20 min)	*L. japonica*	5.5–8	35	When the yield reached 60 mL H_2/g dcw, the operation was shifted to continuous mode	[78]
CSTR	Anaerobic digester sludge	Heat shock (90 °C, 10 min)	Food waste	5.3	35	When cumulative H_2 production was equivalent to 0.5 mol H_2/mol hexose, the reactors were put into continuous mode	[15]
CSTR	Anaerobic digester sludge	Heat shock (90 °C, 15 min)	Sucrose	5.3	35	20 days long start-up	[47]
CSTR	Wastewater treating sludge	Heat shock (100 °C, 45 min)	Starch	5.5	35	Continuous feeding started after 24 h of batch operation. During start-up, decreased initial organic loading rate could enhance hydrogen production efficiency	[48]
CSTR	Wastewater sludge	Heat shock (100 °C, 45 min)	Sucrose	6	35	The fermenter was first operated in a batch mode for two days and then switched to a continuous operation	[63]
CSTR	Indigenous microflora of substrate	–	Cheese whey	5.2	35	For start-up, the reactor was operated in batch mode for 24 h to activate the indigenous microflora contained in the seed before initiation of continuous operation	[34]
CSTR	Anaerobic digester sludge	N.M.	Whey permeate	4–5	35–38	Continuous bioreactors were operated as a batch for the first 40 h	[79]
CSTR	Anaerobic digester sludge	Heat and acid treatment (98 °C, 2 h; pH=2, 24 h)	Glucose	5.5	37	1 day in batch mode before continuous operation, Steady gas production was observed after 19 days	[66]
CSTR	Sewage sludge	–	Terephthalic acid processing wastewater	6	35	Stabilized gas production was achieved after 25 days	[46]

N.M.: not mentioned; N.C.: not controlled.

wash out of existing microbes possessing inadequately low growth rates or adaption capabilities [42]. In other words, feeding regime affects the culture diversity and the relative abundance of the bacterial species.

Additionally, pH, temperature and ORP adjustment are also of crucial importance, since their values change the generation time, growth rate and metabolic pathways of microorganisms present in a mixed culture [12,43–45]. It was also demonstrated that sustained continuous hydrogen formation could be achieved with a start-up strategy completely lacking preliminary inocula pretreatment and batch propagation. For example, bioreactor inoculated with untreated consortia achieved the suppression of H_2 consuming microorganisms through the simultaneous enrichment of biohydrogen producers, taking place because of the insistent acidophilic microenvironment maintained from the beginning of operation [46]. For more studies skipping inocula pretreatment, the reader is referred to Tables 1 and 2.

Besides the adequate substrate composition and loading, temperature, pH and OPR there are other parameters such as hydrogen partial pressure in the bioreactor that may need a control since it is a potential threat that hydrogenotrophic consortial activity may be provoked under high H_2 concentrations [25]. From another point of view, reduced H_2 partial pressure was proven to increase hydrogenase activity and making H_2 formation thermodynamically favorable [47].

3.3. Switch-over time

Since the establishment of continuous hydrogen fermentation implicates an initiative batch cycle for most cases, another issue to be discussed is its duration.

The literature is not consistent about this question, or in other words, it is not fully obvious when to convert to continuous hydrogen fermentation. However, as listed in Tables 1 and 2, the following strategies could be identified as the most popular ones:

- switch-over when significant biohydrogen production commences,
- switch-over when reaching the exponential H_2 production phase, and
- switch-over after a few days of batch cultivation.

Regardless the hydrogen fermentation system employed, dilution rate, substrate loading intensity, pH and temperature applied during transition-state (caused by the switch between batch and continuous operation) reactor run will result in the enrichment of certain bacterial populations and moreover, these factors inherently direct their metabolic pathways. After a period of time when the functional consortia got used to the environmental conditions and consequently stabilized, steady-state can take place which is mostly considered to reach when variations in H_2 gas production, pH and effluent (spent media) quality

Table 2
Start-up experiences during H_2 fermentation in reactors other than CSTR.

Reactor type	Inoculum	Inoculum Pretreatment	Substrate	pH	T	Start-up experiences	Reference
UASBR	Mixture of precultured and granulated sludge	Heat shock (100 °C, 2 h)	Starch	5	55	After confirming the exponential production of the biogas, the operation was turned into continuous mode	[73]
UASBR	Sewage sludge	Heat shock (100 °C, 45 min)	Sucrose	6.7	35	Time-consuming start-up, almost 40 days were taken to reach steady-state	[52]
AFBR	Wastewater treating sludge	Heat shock (90 °C, 15 min)	Glucose, cheese whey	N.M.	30	Initially, the reactors were operated as batch for 76 h prior to switching to continuous mode	[29]
UASBR, CSTR	Anaerobic digester sludge	Heat shock (90 °C, 20 min)	Coffee drink manufacturing wastewater	5.5	35	Continuous operation was delayed until a yield of 0.5 mol H_2/mol hexose achieved in the batch, start-up took 10 days in continuous mode	[77]
UASBR	Anaerobic digester sludge	Heat shock (90 °C, 20 min)	Coffee drink manufacturing wastewater	5.5	35	When the yield of produced H_2 reached 0.5 mol H_2/mol hexose, continuous operation started	[59]
ASBR	Anaerobic digester sludge	Heat shock (90 °C, 10 min)	Food waste	5.3	35	When cumulative H_2 production of 0.5 mol H_2/mol hexose was observed, the reactors were put into continuous operation. Steady-state was reached in 10–30 days depending on the HRT and inoculation conditions	[57]
AFBR	Wastewater treating sludge	Heat shock (90 °C, 10 min)	Glucose	N.C.	30	The bioreactor was initially run as a batch for 2 days to stimulate the hydrogen-producing biomass	[16]
ABR	Anaerobic digester sludge	Heat shock (105 °C, 2 h)	Tapioca wastewater	6.5[a]; 9[b]	32	Multistep batch operation and gradual acclimatization of mixed consortia to substrate. First 3 days in batch operation. 37 days were required to reach steady-state H_2 production	[64]
ASBR	Anaerobic digester sludge	Heat shock (100 °C, 30 min)	Liquid swine manure mixed with glucose	5	37	Firstly, the bioreactor was operated in a batch mode for 24 h until the established biogas production took place	[56]
ASBR	Anaerobic digester sludge	Heat shock (boiled, 30 min)	Liquid swine manure mixed with glucose	5[a]; 4.4–5.6[b]	37	Firstly, the bioreactor was operated in a batch mode for 24 h until established biogas production took place	[58]
UASBR	Enriched facultative anaerobic culture	Enriched culture with *Clostridium pasteurianum*	Citric acid wastewater	7	35–38	More than a month long acclimatization before starting continuous mode. UASB start-up took 20 days, excellent system stabilization	[55]
UASBR	Wastewater treating sludge	N.M.	Sucrose	6.1–9.5	39	The start-up of the UASB reactor lasted for 300 days to enrich H_2-producing microbes and establish a stable gas generation. Afterwards, a successful operation was achieved with the formation of the H_2-producing granules	[53]

ABR: anaerobic baffled reactor; AFBR: anaerobic fluidized bed reactor; N.M.: not mentioned; N.C.: not controlled.

[a] During start-up.
[b] After start-up.

e.g. in terms of soluble metabolic product (SMP) distribution and related concentrations are below 10% on a daily average base [48].

Therefore, appropriate threshold levels of the parameters mentioned can develop an attractive hydrogen-generating bio-community and govern the whole bioreactor towards better performances e.g. volumetric production rates and yields [49].

3.4. Bioreactor configuration

The configuration of the bioreactor set-up is also a concern to keep in mind since different kinds of reactors can be characterized by distinct start-up stage features, for example in terms of its duration [6,14]. Nowadays, the suspended-cell, completely stirred tank reactor (CSTR) is the most routinely applied one, however, up-flow anaerobic sludge blanket (UASBR) reactors, anaerobic membrane bioreactors and immobilized (e.g. fluidized bed) bioreactors [16,29] became popular due to their improved H_2 producing potentials.

Generally, the CSTR is featured by a relatively short induction phase [50] as compared to other applications (e.g. UASBR) due to better mass transfer, but it needs rigorous supervision due to the disposition of cells to wash out at inadequate operating bioreactor regimen. Troubleshooting the risk of wash out can be performed by biomass-rejective systems such as the membrane bioreactors [6] or immobilized e.g. fixed bed systems [51,70]. These alternatives may demonstrate more robust operation and enhanced hydrogen production efficiency even in smaller reactor volumes that is of economical importance.

Another option is the usage of UASBR. Basically, this construction is described by extended start-up phase [52,53] since the flocculation of bacterial communities in the sludge-bed demands longer time. However, start-up period of granular systems for biohydrogen generation could be considerably shortened through the transformation of methanogenic granules (obtained e.g. from already existing and well-established anaerobic, methane forming UASB reactors) into hydrogen producing ones, as recently reported [54].

An important trait of UASBR is the fact that it does not apply mechanical mixing and therefore pH gradients can occur which is not easy to control. For pH regulation purposes, the buffer capacity of the fermentation media may be adjusted to withstand progressive pH depression caused by the formation of acidic by-products that is always expectable in parallel to H_2 bioproduction. However, in return to laborious start-up, granulated reactors reflect remarkably improved operational stability [55]. This is because granulation reactors enhance the active biomass concentration and thereby able to sustain under increased substrate dosage and greater dilution rates so that higher H_2 production intensity can be accomplished [56].

Last but not least, the anaerobic sequencing batch reactor is also among the available design options for continuous H_2 fermentation [56–58].

4. Experiences and lessons of continuous H_2 producing bioreactor start-up

Alternatively to the strategy described in [54], it has been reported that the start-up time of UASBR could also be significantly decreased by thriving H_2-producing cells in CSTR arrangement prior to transferring them to the up-flow anaerobic sludge blanket reactor as seeding source [59]. As it was found, despite the high shearing forces in CSTR, self-flocculation of hydrogen-generating bacteria was notably faster than in UASBR and explained by the more intense mass transfer capacity of the former reactor type.

Besides, organic loading rate fluctuation was reported to accelerate the start-up process both in suspended- and immobilized cell applications [31]. This strategy was shown as an efficient way to rapidly and effectively establish a good biohydrogen evolver culture and less than 3 weeks were required to obtain stabilized operation, which is considerably shorter in comparison with other similar systems [60].

In relation to start-up duration, anaerobic sequencing batch reactor (ASBR) was demonstrated as a feasible concept to provide quick steady-state. Studies indicated that start-up time requirements in the range of 12–14 days were far below the values revealed for UASBR and CSTR [56,61].

As communicated [62], operational conditions employed in UASBR start-up could have significant impact on the microbial fingerprint of mixed H_2 producing sludge, depending on the seed inocula structure.

Likewise, pH adjustment was noticed to express marked influence during H_2-formation bioreactor start-up [46,58]. It has appeared that pH values out of optimal range may induce population- and metabolic shifts (i.e. solventgenesis). Moreover, the hydrogenase enzyme activity and growth rate of microorganism responsible for H_2 fermentation can also be hindered under inappropriate pH conditions. Hence, non-optimal pH probably causes an unambiguous delay in attaining steady-state conditions with the desired H_2 production rate and yield.

Moreover, pre-culturing seed inocula and initial batch strategy were observed to be efficient start-up concept that supported biomass growth and consistent H_2 production well in subsequent continuous operation [63]. The adaption of H_2 producing microflora to a given substrate can be conducted in complex and multistep batch operation, where bacterial consortia are periodically supplied with gradually increasing feed concentrations before putting into continuous mode [64]. Nevertheless, as mentioned above, there are studies that skipped preliminary batch operation and adapted the H_2 generating cultures during continuous operation. Regarding these start-up strategies, it is to conclude that the hydraulic retention time (HRT) is usually stepwise refined from long to short time intervals to allow the acclimatization of microorganisms to new environments and prevent washing out the bacteria of interest [65]. As a result of shifting HRT, the microbial population dynamically changes leading to the disappearance of certain species while others show up [66].

Additionally to HRT, altering organic loading rate (OLR) is also a stress source to the strains that are forced to get accustomed to new surroundings [52]. Varying OLR might cause sporulation in hydrogen producing cultures and contribute to the observable fluctuations in the H_2 production efficacy during start-up stage [48,67].

It is an ongoing progress in biohydrogen research that various waste substances are utilized to improve process viability. However, depending on the nature of these problematic raw materials (e.g. cheese whey), it is presumptive that they contain indigenous microflora which of course, from the beginning, might affect bioreactor performance in a negative manner [75]. In this regard, it was evaluated [15] that attention should be paid to the pretreatment (e.g. by means of alkali) and sufficient storage circumstances (preferentially at cold temperature) of such streams. This is attributed to the fact that indigenous strains, for example non-H_2-producing acidogens such as lactic- and propionic bacteria present in non-aseptic substrate could cause strong contamination and even outcompete the H_2-producing microbes [68,69].

Even though the biosystems may resist the perturbations caused by contamination and might be able to express the performance required, dominance of disadvantageous non-hydrogen producing bacteria potentially leads to problematic start-up, unsuccessful continuous operation in long terms and consequently may force to reinoculate and restart the H_2 fermenting bioreactor.

Recently, it was experimentally demonstrated that non-hydrogen producing cells, as a result of long batch cultivation, were promoted alongside their useful H_2 evolver counterparts and could take over, causing tough start-up failure. In order to avoid such undesired microbiological activity, an early switch-over time was recommended that highly increased the chance of successful and long-term continuous H_2 fermentation [69]. It is also extractable from the literature that inocula source can be a determining factor of the time necessary for starting-up a continuous H_2 producing fermenter [70].

In addition to contamination related issues, operational failures caused by unforeseen technical difficulties (e.g. broken pumps, leaking tubes) may also challenge the adequate start-up since they can cause insufficient or shocking organic loading rates, altered hydraulic detention times and thus, disturb the developing microbial consortia and affect its survivability.

To aid bioreactor start-up, monitoring the soluble metabolites such as volatile fatty acids is apparently beneficial. The ratios of acetic-, butyric-, and propionic acids produced by the various groups of microorganisms can be useful feedbacks about the state of the hydrogen producing consortia. When propionic acid concentration gradually increases in fermentation broth, it assumes the occurrence of microbes with no utility for H_2 production and gives the sign that troubleshooting of the reactor is required to avoid strong deterioration of its performance.

In cases when an unusual decline in hydrogen production efficiency occurs (e.g. as a consequence of population shift or appearance of methanogenic activity) during start-up that threats achieving steady-state, a temperature shift strategy may be carried out which includes heating the bioreactor to higher temperature ranges (e.g. to 70–80 °C) for a short time (e.g. 1 h) to reclaim hydrogen producing bacteria and reactor performance [71]. However, it may be ineffective for granulated systems, since it was proven that granules serve as protective structure. In such cases, disintegration of granules prior to temperature shift or combined methods (e.g. temperature- and pH shift together) might work [62]. On the contrary, it was demonstrated that simple washing and subsequent boiling of granular sludge could be a feasible approach to inactivate hydrogen consuming microorganisms [49].

It is to mention that process temperature – even in the same bioreactor design – could influence the reactor behavior during start-up. As a matter of fact, comparison of meso- and (hyper) thermophilic H_2 production in CSTRs indicates that start-up of the latter group could last as long as 90 days to achieve stabilized H_2 fermentation [72], while mesophilic H_2 production in similar system configurations was reported to reach steady-state circumstances in shorter times [39,47,67].

As a summary, the flow chart depicted in Fig. 1 presents the connection network of the various steps involved in continuous hydrogen producing bioreactor start-up.

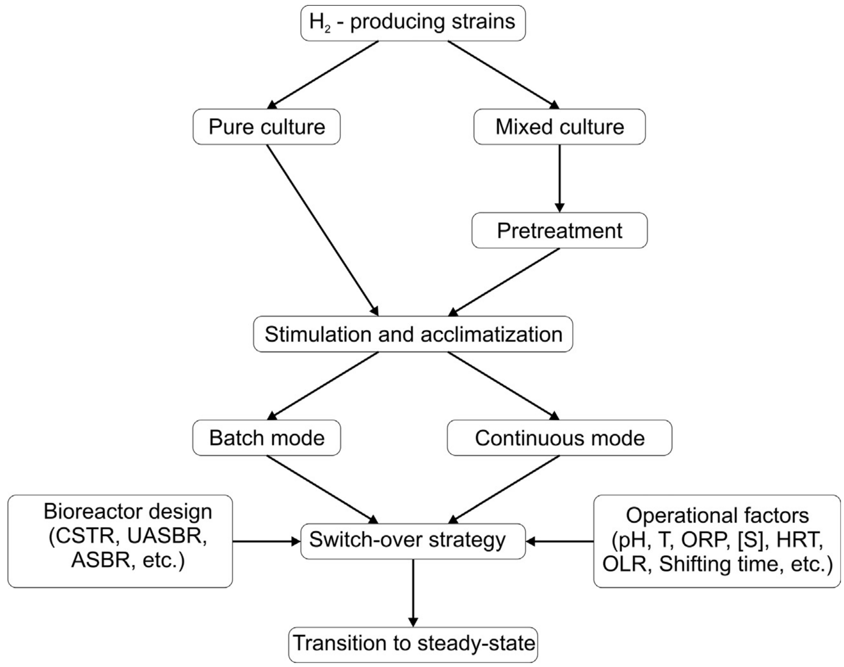


Fig. 1. Flow chart illustrating the various steps of start-up process.

5. Conclusions

In this review, the experiences of continuous hydrogen fermentation start-up were scoped and analyzed. The lessons of relevant literature papers about the routes leading to continuous and efficient, steady-state hydrogen production imply that start-up is of high concern to avoid significant performance losses. As a general guideline, the establishment of reliable, continuous hydrogen producing bioreactors should start with proper inocula selection and its pretreatment (if necessary), followed by an acclimatization period – conducted mainly as a batch – to adopt the living biocatalysts to the intended substrate which may also require preliminary treatment to eliminate native and undesirable microflora present in it. Subsequently, switch-over strategy – assigned to ensure viable and smooth batch to continuous shift – must be designed e.g. timed properly in order to preserve and ensure microorganism with as high hydrogen producing capacity as possible. Besides timing, transition from batch to continuous mode hydrogen fermentation should take also into account the suitable adjustment of major environmental (physiological) factors – such as pH, temperature, etc. – and the operating conditions (e.g. hydraulic retention time, organic loading rate, etc.) applied with respect to bioreactor configuration (CSTR, UASB, AnMBR, etc.). Currently, CSTRs are the most widely used reactors for continuous hydrogen production due to their relatively rapid start-up phase. Nevertheless, as a result of the efforts made to cut start-up time demand of other devices, more wide-spread employment of granular- and immobilized systems and that of reactors integrated with downstream (membrane bioreactors) is presumable in the future, which is also attributed to their potential benefits (e.g. higher long-term performance and enhanced stress tolerance) over the conventional set-ups.

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